JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2007, p. 4006–4010 Vol. 45, No. 12
0095-1137/07/$08.00+0 doi:10.1128/JCM.00740-07

Changing Antimicrobial Susceptibility Epidemiology of *Helicobacter pylori* Strains in Japan between 2002 and 2005[▽]

Intetsu Kobayashi,[1]* Kazunari Murakami,[2] Mototsugu Kato,[3] Seiichi Kato,[4] Takeshi Azuma,[5] Shin'ichi Takahashi,[6] Naomi Uemura,[7] Tsutomu Katsuyama,[8] Yoshihiro Fukuda,[9] Ken Haruma,[10] Masaru Nasu,[11] and Toshio Fujioka[12]

Chemotherapy Division, Mitsubishi Chemical Medience Corporation, 3-30-1 Shimura Itabashi-ku, Tokyo 174-8555, Japan[1]; Department of Gastroenterology, Oita University Faculty of Medicine, 1-1 Idaiga-oka Hasama-machi, Oita 879-5593, Japan[2]; Division of Endoscopy, Hokkaido University Hospital, Kita-14-jyo Nishi-7-chome, Kita-ku Sapporo-shi, Hokkaido 060-0814, Japan[3]; Department of Pediatrics, Tohoku University School of Medicine, 1-1 Seiryo-machi Aoba-ku Sendai 980-8574, Japan[4]; Department of Gastroenterology, Kobe University School of Medicine, 7-5-2 Kusunoki-cho Chuo-ku Kobe-shi, Hyogo 650-0017, Japan[5]; Department of Internal Medicine, Kyorin University School of Medicine, 6-20-2 Shinkawa Mitaka-shi, Tokyo 181-8611, Japan[6]; Department of Gastroenterology, International Medical Center of Japan, 1-21-1 Toyama Shinjuku-ku, Tokyo 162-8655, Japan[7]; Laboratory Medicine, Shinshu University Graduate School of Medicine, 3-1-1 Asashi Matsumoto-shi, Nagano 390-8621, Japan[8]; Clinical Nutrition and Health Science, Hyogo College of Medicine, 1-1 Mukogawacho, Nishinomiya-shi, Hyogo 663-8501, Japan[9]; Division of Gastroenterology, Department of Internal Medicine, Kawasaki Medical School, 577 Matsushima Kurashiki-shi, Okayama 701-0192, Japan[10]; General Clinical Research Center, Oita Nakamura Hospital, 3-2-43 Ote-machi, Oita 870-0022, Japan[11]; and Department of Gastroenterology, Faculty of Medicine, Oita University, 1-1 Idaiga-oka Hasama-machi, Oita 879-5593, Japan[12]

Received 5 April 2007/Returned for modification 3 July 2007/Accepted 4 October 2007

Surveillance of *Helicobacter pylori* antimicrobial susceptibility reflecting the general population in Japan is limited. The antimicrobial susceptibilities of 3,707 *H. pylori* strains isolated from gastric mucosa samples of previously untreated patients diagnosed with gastroduodenal diseases at 36 medical facilities located throughout Japan between October 2002 and September 2005 were evaluated. Using an agar dilution method for antimicrobial susceptibility testing of *H. pylori*, the MIC distributions and trends during the study period for clarithromycin, amoxicillin, and metronidazole were studied. While the MIC_{50} and MIC_{90} for clarithromycin did not change during the 3-year period, the MIC_{80} showed a 128-fold increase. Furthermore, the rate of resistance increased yearly from 18.9% (2002 to 2003) to 21.1% (2003 to 2004) and 27.7% (2004 to 2005). With a resistance rate of 19.2% among males compared to 27.0% among females, a significant gender difference was observed ($P < 0.0001$). Our study shows that in Japan, there is an evolving trend towards increased resistance to clarithromycin with geographical and gender differences as well as between clinical disease conditions. No significant changes in resistance were observed for amoxicillin and metronidazole during the period. While the benefit of *H. pylori* antimicrobial susceptibility testing has been debated in Japan, current empirical regimens are not based on susceptibility data representative of the general population. The development of an effective *H. pylori* eradication regimen in Japan will require continued resistance surveillance as well as a better understanding of the epidemiology of resistance.

Since the report of Marshall and Warren in 1984 (11a) implicating *Helicobacter pylori* in stomach ulcer and gastritis, *H. pylori* has been implicated in not only peptic ulcer disease but also stomach cancer (23). The incidence of stomach cancer in Japan has been reported to be six to nine times higher than that in the United States (18, 22). As stomach cancer is a major cause of mortality in Japan, *H. pylori* eradication is an important public health intervention measure.

H. pylori eradication is based on a combination of antimicrobials and antisecretory agents (21). Although triple-therapy regimens that include clarithromycin (CLR) remain the most widely used first-line treatment for *H. pylori* infection, with eradication rates estimated to be 80%, eradication rates are believed to be falling in Japan as they are in other countries (2, 11, 26). Causation factors related to declining eradication rates include increasing resistance as a result of mutation of the 23S rRNA gene, poor patient compliance, and differences reflecting genotypes of CYP2C19 (1, 7, 11). Of the factors contributing to declining eradication rates, resistance is considered to be the most important one.

Current national health care reimbursement in Japan covers testing and therapy related to *H. pylori*-associated peptic ulcers. As of November 2000, for reimbursement purposes, the Japanese national health care insurance system established a standard of a triple-drug therapy consisting of a proton pump inhibitor (PPI), CLR, and amoxicillin (AMX). Since the inclu-

* Corresponding author. Mailing address: Chemotherapy Division, Mitsubishi Chemical Medience Corporation, 3-30-1 Shimura Itabashi-ku, Tokyo 174-8555, Japan. Phone: 81-3-5994-2334. Fax: 81-3-5994-2939. E-mail: mbc-ka@sa2.so-net.ne.jp.

▽ Published ahead of print on 17 October 2007.

sion of an *H. pylori* eradication regimen in the national health care reimbursement system, there has been considerable debate among health care experts in Japan regarding how to best deal with the difficulties of eradication. This debate has intensified in light of growing resistance to antimicrobial agents and the all-too-common relapse observed clinically in the treatment of gastroduodenal ulcers.

Resistance rates in *H. pylori* can vary between groups of patients according to age, sex, disease, and place of residence (6). Previous antimicrobial susceptibility surveillance studies monitoring antimicrobial resistance in *H. pylori* in Japan have been limited in scope, and there is no systematic surveillance of primary antimicrobial resistance. In the face of increasing eradication failure in Japan linked to growing resistance to CLR, we undertook a broad, population-based survey of *H. pylori* antimicrobial susceptibilities. In addition to CLR, we included AMX, which has shown low rates of resistance in previous limited population studies, as well as metronidazole (MNZ), a commonly used antimicrobial agent used outside of Japan. With an antimicrobial susceptibility database reflecting a broad cross section of the Japanese population, the data will be useful in supporting efforts to develop an *H. pylori* eradication regimen reflecting accurate resistance patterns and epidemiologies.

MATERIALS AND METHODS

Patients and bacterial strains. *H. pylori* isolates were collected sequentially from patients with no history of antibacterial therapy for eradication. These patients ranged in age from 6 to 88 years, were diagnosed with gastroduodenal disease, came from 36 medical facilities representing different geographical regions of Japan, and were included in the study on the basis of chronological and geographical considerations. A total of 3,707 isolates were included in the study: 1,069 isolates from October 2002 to September 2003, 1,381 isolates from October 2003 to September 2004, and 1,257 isolates from October 2004 to September 2005. Only one isolate per patient was included in the study.

H. pylori isolates used in this study were identified on the basis of colonial morphology, characteristic spiral morphology on Gram staining, and other positive findings as described previously (25). Isolates were stored at −70°C in *Brucella* broth with 10% dimethyl sulfoxide and 10% horse serum. Only one *H. pylori* isolate per patient was included in the study.

Antimicrobial susceptibility testing. Antimicrobial susceptibility was determined by the CLSI agar dilution method as previously described (4, 5). Briefly, after adjusting for potency, twofold dilutions of CLR (LKT Labs, MN), AMX (Sigma-Aldrich, MO), and MNZ (Sigma-Aldrich) were prepared in Mueller-Hinton agar (Becton Dickinson, MD) with 5% sheep blood. Following preparation an *H. pylori* suspension in physiological saline adjusted to a McFarland standard of 2.0, a 1- to 3-μl inoculum was spotted onto each agar plate. Following incubation at 35°C ± 2°C for 72 h under a microaerophilic atmosphere, the MICs of CLR and AMX were established as the drug concentration showing no growth. With respect to the MIC of MNZ, the MIC was the concentration at which a marked reduction in the appearance of growth on the antimicrobial agent-containing plate compared to growth on the antimicrobial-free control plate occurred; significant changes in growth included a change from confluent growth to a haze, less than 10 tiny colonies, or one to three normal-sized colonies. Quality control using *H. pylori* ATCC 43504 was included with each antimicrobial susceptibility test.

The MIC interpretive standard of CLR for *H. pylori* (resistant, ≥1 μg/ml) established by the CLSI was used (5). For AMX, the interpretive standard (susceptible, ≤0.03 μg/ml) established by the Japanese Society of Chemotherapy was used (8). As MNZ is not routinely used in Japan and no CLSI interpretive criteria exist, MICs were generated for trending purposes.

Statistical analysis. Results for the years 2002 to 2005 were determined by calculating data specific for each year and then averaging the data. Data on CLR resistance rates were stratified by age. Data were analyzed statistically using the chi-square test, and a P value of <0.05 was considered to be statistically significant.

RESULTS

The distribution of *H. pylori* MICs of CLR, AMX, and MNZ over a 3-year period is shown in Table 1.

Interestingly, CLR MICs showed a bimodal distribution centering on 0.03 to 0.06 μg/ml and 16 to 32 μg/ml. With respect to CLR, the MIC range did not broaden over the study period. While the MIC_{50} and MIC_{90} during this period did not show a significant rate of change in resistance, the MIC_{80} revealed a significant yearly increase as shown by a 128-fold increase during the 3-year period. Moreover, the overall rate of resistance to CLR increased from 18.9% (2002 to 2003) to 27.7% (2004 to 2005) ($P < 0.0001$).

With respect to AMX, the distribution of MICs during the 3-year period remained constant, with 61.8 to 68.8% of the isolates demonstrating MICs of ≤0.015 μg/ml. MIC_{50} and MIC_{90} values also remained constant during the period. There were only three isolates at the upper end of the MIC distribution of 2 μg/ml. No isolate exhibited an MIC of ≥4 μg/ml. Using the resistance breakpoint established by the Japanese Society of Chemotherapy, no significant trend toward resistance was observed.

The MIC distribution for MNZ was characterized by a wide distribution with a mode of 2 μg/ml over the period, with no significant shifts in MIC_{50}, MIC_{80}, or MIC_{90} values.

As shown in Table 2, there was a statistically significant difference in the overall rates of resistance to CLR of 19.2% and 27.0%, respectively, among males and females ($P < 0.0001$); moreover, resistance rates varied when stratified by age range. The differential resistance rates among males and females were most extreme in the interval of 6 to 29 years of age, where resistance rates were 8.7% and 34.0% for males and females, respectively ($P < 0.0001$). Among males, CLR resistance was highest in those 70 years of age and older, at 28.3%, which was significantly higher than the resistant rate of 18.9% seen in the age group of 30 to 49 years ($P < 0.001$). Among females, a statistically significant difference in CLR resistance rates was also seen between groups of patients 70 years of age and older and 30 to 49 years of age ($P < 0.05$).

In Table 3, CLR resistance rates among *H. pylori* isolates recovered from different clinical disease conditions reveal that compared to gastroduodenal ulcer or gastric ulcer, resistance rates were higher in isolates recovered from cases of chronic gastritis ($P < 0.0001$).

In addition to differences in CLR resistance by age, gender, and clinical disease condition, geographical differences were also revealed (Table 4). The Kanto region (11.6%), located in the middle region of Japan, was associated with a lower rate of resistance than the Kansai region (33.3%), located in the Southwest region of Japan ($P < 0.0001$).

Quality control MICs were within acceptable limits for all antimicrobial susceptibility testings.

DISCUSSION

Following a 1994 National Institutes of Health Consensus Development Conference recommendation that peptic ulcer patients with *H. pylori* infection be treated with antimicrobial agents in addition to antisecretory drugs (17), Japan adopted a similar approach in November 2000 after a series of large-scale

TABLE 1. Distribution of *H. pylori* MICs over a 3-year period

Agent	Period (yr)	Total no. of isolates	No. of isolates with MIC (μg/ml) (% cumulative distribution) of: ≤0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128	MIC_{50} (μg/ml)	MIC_{80} (μg/ml)	MIC_{90} (μg/ml)	% Resistant strains
CLR	2002–2003	1,069	244 (22.8)	283 (49.3)	257 (73.3)	72 (80.0)	10 (81.0)	1 (81.1)		1 (81.2)	9 (82.0)	43 (86.1)	52 (90.1)	59 (96.4)	27 (99.0)	11 (100.0)		0.06	0.12	16	18.9[a]
	2003–2004	1,381	91 (6.6)	263 (25.6)	531 (64.1)	179 (77.0)	24 (78.8)	1 (78.9)	4 (79.1)	2 (79.3)	20 (80.7)	44 (83.9)	73 (89.2)	90 (95.7)	50 (99.3)	8 (99.9)	1 (100.0)	0.06	4	32	21.1
	2004–2005	1,257	279 (22.2)	313 (47.1)	257 (67.5)	56 (72.0)	4 (72.3)				15 (73.5)	77 (79.6)	118 (89.0)	101 (97.1)	35 (99.8)	2 (100.0)		0.06	16	32	27.7[a]
AMX	2002–2003	1,069	735 (68.8)	172 (84.8)	111 (95.2)	40 (99.0)	7 (99.6)	3 (99.9)	1 (100.0)									≤0.015	0.03	0.06	15.2[b,c]
	2003–2004	1,381	853 (61.8)	232 (78.6)	165 (90.5)	103 (98.0)	20 (99.4)	6 (99.9)		2 (100.0)								≤0.015	0.06	0.06	21.4[b]
	2004–2005	1,257	804 (63.9)	248 (83.7)	136 (94.5)	48 (98.3)	16 (99.6)	4 (99.9)		1 (100.0)								≤0.015	0.03	0.06	16.3[b,c]
MNZ	2002–2003	1,069					2 (0.2)	183 (17.3)	340 (49.1)	423 (88.7)	69 (95.1)	22 (97.2)	9 (98.0)	15 (99.4)	6 (100.0)			2	2	4	—[d]
	2003–2004	1,381						136 (9.8)	478 (44.5)	574 (86.0)	120 (94.7)	27 (96.7)	11 (97.5)	20 (98.9)	15 (100.0)			2	2	4	—[d]
	2004–2005	1,257						188 (15.0)	491 (54.0)	497 (93.6)	39 (96.7)	15 (97.9)	13 (98.9)	7 (99.4)	6 (99.9)	1 (100.0)		1	2	2	—[d]

[a] $P < 0.0001$ (2002–2003 versus 2004–2005).
[b] Percentage of nonsusceptible strains.
[c] No significant difference (2002–2003 versus 2004–2005).
[d] —, no interpretive criteria.

TABLE 2. Distribution of 3,530 *H. pylori* CLR-resistant isolates over a 3-year period by age and gender[a]

Age group (yr)	No. of resistant isolates/total no. of isolates (%): Male	Female
6–29	12/138 (8.7)[b,c,d]	16/47 (34.0)
30–49	97/512 (18.9)[e]	79/267 (29.6)[e]
50–69	202/1,169 (17.3)[f]	182/658 (27.7)
≥70	116/410 (28.3)	74/329 (22.5)
Total	427/2,229 (19.2)	351/1,301 (27.0)[g]

[a] Information on patient gender for 177 isolates could not be retrieved.
[b] $P < 0.05$ (6 to 29 versus 30 to 49 years of age).
[c] $P < 0.05$ (6 to 29 versus 50 to 69 years of age).
[d] $P < 0.05$ (6 to 29 versus ≥70 years of age).
[e] $P < 0.05$ (30 to 49 versus ≥70 years of age).
[f] $P < 0.05$ (50 to 69 versus ≥70 years of age).
[g] $P < 0.05$ (male versus female).

clinical trials. Drugs approved for treatment consisted of a PPI (lansoprazole), AMX, and CLR. Subsequently, omeprazole was substituted for lansoprazole as the choice of PPI in April 2002.

The numbers of patients who were placed under triple-therapy *H. pylori* treatment increased from 270,000 in 2003 to 340,000 in 2005. With the combination of lansoprazole, AMX, and CLR (Takeda Pharmaceuticals, Osaka, Japan), compliance to medication has increased, while errors in medication have decreased (10). As data on the use of the combination of lansoprazole, AMX, and CLR by region in Japan are not available, it has been difficult to correlate drug usage and local resistance patterns. As resistance to antimicrobial agents increases in response to eradication treatment regimens, it has been speculated that resistance pattern differences observed in limited regional studies in Japan may be related to medical practice differences with respect to eradication treatment.

While an eradication success rate of 80% was previously assumed for triple therapy, eradication treatment failure has been a growing concern in recent years. Factors contributing to eradication include reduced compliance to medication schedules, genetic polymorphisms of drug-metabolizing enzymes, and increased resistance to antimicrobial agents (2, 7, 26). It

TABLE 3. Distribution of *H. pylori* CLR-resistant isolates over a 3-year period by disease category[a]

Diagnosis	No. of isolates	No. of resistant isolates (%)
Gastric ulcer	997	200 (20.0)[b]
Gastroduodenal ulcer	226	37 (16.4)[c]
Duodenal ulcer	826	141 (17.1)[d,e]
Chronic gastritis	850	264 (31.1)[f]
Gastric cancer	288	66 (22.9)
MALT	32	6 (18.8)
Total	3,219	714 (22.2)

[a] Information on diagnosis for 488 isolates could not be retrieved. MALT, mucosa-associated lymphoid tissue.
[b] $P < 0.05$ (gastric ulcer versus chronic gastritis).
[c] $P < 0.05$ (gastroduodenal ulcer versus chronic gastritis).
[d] $P < 0.05$ (duodenal ulcer versus chronic gastritis).
[e] $P < 0.05$ (duodenal ulcer versus gastric cancer).
[f] $P < 0.05$ (chronic gastritis versus gastric cancer).

TABLE 4. Distribution of *H. pylori* CLR-resistant isolates over a 3-year period by region

Region	No. of medical sites	No. of isolates	No. of resistant isolates (%)[a]
Hokaido	4	269	57 (21.2)
Tohoku region	6	317	60 (18.9)[a]
Kanto region	8	696	81 (11.6)
Chubu region	5	408	79 (19.4)
Kansai region	2	546	182 (33.3)[b]
Chugoku region and Shikoku	6	674	184 (27.3)
Kyushu and Okinawa	4	797	200 (25.1)
Total		3,707	843 (22.7)

[a] $P < 0.05$ (Tohoku region versus Kanto region).
[b] $P < 0.05$ (Kansai region versus Chugoku region and Shikoku).

was previously reported that resistance to CLR among *H. pylori* isolates in Japan has been increasing (1, 9, 11, 14, 27). It can be speculated that inadequate empirical treatment due to a lack of current antimicrobial susceptibility data is exacerbating the problem of increasing resistance. A survey conducted in 2000 by the Japanese Society of Chemotherapy found the national resistance rate for CLR to be 7.0%, while a similar survey in 2004 found a 27.7% resistance rate. Similar studies outside of Japan found lower rates. In 2004, the CDC reported 13% CLR resistance and warned of the potential for eradication failure (6). A possible reason accounting for the significantly higher rate of resistance to CLR in Japan is the more frequent use of macrolides in the practice of pulmonary and ear, nose, and throat specialties, as macrolides can be prescribed regardless of patient age on an outpatient basis (19). In particular, the relative clinical effectiveness of low-dose long-term therapy with macrolides in the treatment of chronic respiratory infections may account for the failure of *H. pylori* eradication in those patients.

The high failure rate observed when CLR is employed in an eradication regimen in the presence of CLR-resistant strains as well as the association of eradication failure and CLR resistance have been reported (9, 27). The not-uncommon occurrence of suboptimal eradication therapy would predict a further increase in the incidence of CLR-resistant *H. pylori* strains.

In order to determine the current antimicrobial susceptibility profiles of *H. pylori* isolates from gastric and duodenal diseases in Japan, we undertook a surveillance study as part of a working group of the Japanese Society for *Helicobacter* Research. Our findings reveal that resistance to CLR increased from 18.9% to 27.7% over a 3-year period beginning in 2002, along with a sharp rise in the MIC_{80}. This increased resistance to CLR was not detected with the MIC_{50} and MIC_{90} during the 3-year period, while the MIC_{80} reflected the cumulative distribution within the bimodal distributions.

While the number of isolates that were not susceptible to AMX showed a peak during the second-year range of the study, our isolates were below the upper MIC of 8 μg/ml as previously reported by van Zwet et al. (24). While resistance to AMX is believed to be due to a mutation in the gene coding for penicillin binding protein, no *H. pylori* isolates in Japan have, to date, demonstrated a similar mechanism of resistance. This may reflect a different mechanism underlying the elevation of AMX MICs for isolates recovered in Japan compared to that for isolates recovered in the United States and Europe.

Using the MNZ breakpoint of 8 μg/ml established by the European Study Group, resistance rates were 4.9%, 5.3%, and 3.3% during the study period range of 2002 to 2003, 2003 to 2004, and 2004 to 2005, respectively. Compared to data generated in Europe, the resistance rates observed in our study were lower (12). One possible reason to account for this difference may be that for Japanese national health care reimbursement purposes, the use of MNZ is currently restricted to use as an antiprotozoal drug, resulting in less exposure for the development of antibacterial resistance.

In this epidemiological study of the distribution of *H. pylori* antimicrobial resistance in the Japanese population, there are a number of global implications of our findings. With respect to Japan, the use of CLR for respiratory infections has increased significantly. We feel that there is a causal relationship between the increased use of CLR and increased resistance of *H. pylori* to CLR. Another factor specific to Japan is that the use of MNZ is limited to protozoan infections; this is reflected in the high susceptibility of *H. pylori* to this antimicrobial observed in this study. In contrast, there is high rate of *H. pylori* resistance to MNZ in Vietnam as well as other countries in Asia, which may be related to the greater use of this antimicrobial. It can be speculated that the eradication of *H. pylori* will be influenced by the use and abuse of MNZ.

While the implementation of an *H. pylori* eradication regimen in Japan was delayed compared to those in the United States and Europe, our data on present resistance rates clearly suggest that first-line eradication failure as well as resistance to CLR will continue to increase in Japan. The effectiveness of a second-line *H. pylori* eradication regimen consisting of PPI in combination with AMX and MNZ has been reported in Japan, with eradication success rates ranging from 80 to 90% (15, 16). As confirmed by our study, the low rate of resistance to MNZ will most likely lead to the greater use of MNZ in eradication regimens in Japan.

With respect to resistance rates in *H. pylori* between groups of patients varying according to gender, McMahon et al. and Pilotto et al. previously reported higher rates of resistance to MNZ among women with a previous history of MNZ medication (13, 20). On the other hand, no statistically significant difference among the sexes for CLR resistance was reported (13). In our surveillance study, a higher rate of CLR resistance among women was observed over the 3-year period. It can be speculated that this observation may reflect the fact that women in Japan have a greater tendency to seek treatment for relatively minor respiratory infections in light of their role as the primary parent in child-rearing as well as the widespread use of CLR in the empirical treatment of respiratory infections. The hypothesis of higher resistance in patients with a history of exposure to the antimicrobial agent is consistent with a previous report from Bulgaria, which found that among patients receiving treatment for peptic ulcers, *H. pylori* MNZ resistance rates were twofold higher among adults than among children (3). Among minors, MNZ resistance rates were lower in the 1- to 9-year-old age group compared to 10- to 18-year-old age group. In the same study, ciprofloxacin resistance was 8% among the 10- to 18-year-old age group, compared to no resistance in the 1- to 9-year-old age group. The data are

clearly consistent with the association of resistance rates and antibiotic usage.

Finally, the finding of an association of CLR resistance when patients were stratified by disease has not been previously reported in Japan. The significantly higher rate of resistance among patients with chronic gastritis may reflect the use of CLR in treating non-gastritis-related infections in these patients. Our group will continue ongoing research to better understand the epidemiology of *H. pylori* antimicrobial resistance in Japan.

REFERENCES

1. **Alarcon, T., D. Domingo, and M. Lopez-Brea.** 1999. Antibiotic resistance problems with *Helicobacter pylori*. Int. J. Antimicrob. Agents **12:**19–26.
2. **Asaka, M., K. Satoh, K. Sugano, T. Sugiyama, S. Takahashi, Y. Fukuda, H. Ota, K. Murakami, K. Kimura, and T. Shimoyama.** 2001. Guidelines in the management of *Helicobacter pylori* infection in Japan. Helicobacter **6:**177–186.
3. **Boyanova, L., R. Koumanova, G. Gergova, M. Popova, I. Mitov, Y. Kovacheva, S. Derejian, N. Katsarov, R. Nikolov, and Z. Krastev.** 2002. Prevalence of resistant *Helicobacter pylori* isolates in Bulgarian children. J. Med. Microbiol. **51:**786–790.
4. **Clinical and Laboratory Standards Institute.** 2006. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard—7th edition. M7-A7. Clinical and Laboratory Standards Institute, Wayne, PA.
5. **Clinical and Laboratory Standards Institute.** 2006. Performance standards for antimicrobial susceptibility testing. Sixteenth informational supplement M100-S16. Clinical and Laboratory Standards Institute, Wayne, PA.
6. **Duck, W. M., J. Sobel, J. M. Pruckler, Q. Song, D. Swerdlow, C. Friedman, A. Sulka, B. Swaminathan, T. Taylor, M. Hoekstra, P. Griffin, D. Smoot, R. Peek, D. C. Metz, P. B. Bloom, S. Goldschmidt, J. Parsonnet, G. Triadafilopoulos, G. I. Perez-Perez, N. Vakil, P. Ernst, S. Czinn, D. Dunne, and B. D. Gold.** 2004. Antimicrobial resistance incidence and risk factors among *Helicobacter pylori*-infected persons, United States. Emerg. Infect. Dis. **10:**1088–1094.
7. **Furuta, T., N. Shirai, M. Sugimoto, A. Nakamura, A. Hishida, and T. Ishizak.** 2005. Influence of CYP2C19 pharmacogenetic polymorphism on proton pump inhibitor-based therapies. Drug Metab. Pharmacokinet. **20:**153–167.
8. **Japan Society for Chemotherapy.** 2000. Antimicrobial Susceptibility Subcommittee on *Helicobacter pylori* MIC breakpoints for clarithromycin and amoxicillin. Jpn. Soc. Chemother. **48:**561–567.
9. **Kato, M., Y. Yamaoka, J. J. Kim, R. Reddy, M. Asaka, K. Kashima, M. S. Osato, F. A. El-Zaatari, D. Y. Graham, and D. H. Kwon.** 2000. Regional differences in metronidazole resistance and increasing clarithromycin resistance among *Helicobacter pylori* isolates from Japan. Antimicrob. Agents Chemother. **44:**2214–2216.
10. **Kawai, T., K. Kawakami, F. Moriyasu, and Y. Takagi.** 2005. *H. pylori* eradication package set (Lansap). Nippon Rinsho **63**(Suppl. 11)**:**361–365.
11. **Lee, J. H., J. H. Shin, I. H. Roe, S. G. Sohn, J. H. Lee, G. H. Kang, H. K. Lee, B. C. Jeong, and S. H. Lee.** 2005. Impact of clarithromycin resistance on eradication of *Helicobacter pylori* in infected adults. Antimicrob. Agents Chemother. **49:**1600–1603.

11a. **Marshall, B. J., and J. R. Warren.** 1984. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet **i:**1311–1315.

12. **McLoughlin, R., and C. O'Morain.** 2005. Effectiveness of antiinfectives. Chemotherapy **51:**243–246.
13. **McMahon, B. J., T. W. Hennessy, J. M. Bensler, D. L. Bruden, A. J. Parkinson, J. M. Morris, A. L. Reasonover, D. A. Hurlburt, M. G. Bruce, F. Sacco, and J. C. Butler.** 2003. The relationship among previous antimicrobial use, antimicrobial resistance, and treatment outcomes for *Helicobacter pylori* infections. Ann. Intern. Med. **139:**463–469.
14. **Murakami, K., and T. Fujioka.** 2005. Drug resistant *H. pylori* in Japan: general remarks. Nippon Rinsho **63**(Suppl. 11)**:**198–202.
15. **Murakami, K., R. Sato, T. Okimoto, M. Nasu, T. Fujioka, M. Kodama, and J. Kagawa.** 2003. Efficacy of triple therapy comprising rabeprazole, amoxicillin and metronidazole for second-line *Helicobacter pylori* eradication in Japan, and the influence of metronidazole resistance. Aliment. Pharmacol. Ther. **17:**119–123.
16. **Nagahara, A., H. Miwa, R. Ohkura, T. Yamada, K. Sato, M. Hojo, and N. Sato.** 2001. Strategy for retreatment of therapeutic failure of eradication of *Helicobacter pylori* infection. J. Gastroenterol. Hepatol. **16:**613–618.
17. **NIH.** 1994. *Helicobacter pylori* in peptic ulcer disease. NIH Consens. Statement **12:**1–23.
18. **Parkin, D. M., S. L. Whelan, J. Ferlay, L. Teppo, and D. B. Thomas.** 2002. Cancer incidence in five continents. IARC Sci. Publ. **155:**258–269.
19. **Perez Aldana, L., M. Kato, S. Nakagawa, M. Kawarasaki, T. Nagasako, T. Mizushima, H. Oda, J. Kodaira, Y. Shimizu, Y. Komatsu, R. Zheng, H. Takeda, T Sugiyama, and M. Asaka.** 2002. The relationship between consumption of antimicrobial agents and the prevalence of primary *Helicobacter pylori* resistance. Helicobacter **7:**306–309.
20. **Pilotto, A., M. Rassu, G. Leandro, M. Franceschi, F. Di Mario, et al.** 2000. Prevalence of *Helicobacter pylori* resistance to antibiotics in Northeast Italy: a multicentre study. Ulcer. Dig. Liver Dis. **32:**763–768.
21. **Rauws, E. A., and G. N. Tytgat.** 1990. Cure of duodenal ulcer associated with eradication of *Helicobacter pylori*. Lancet **335:**1233–1235.
22. **Ries, L. A. G., M. P. Eisner, and C. L. Kosary.** 2003. SEER cancer statistics review, 1975–2000. National Cancer Institute, Bethesda, MD.
23. **Suerbaum, S., and P. Michetti.** 2002. *Helicobacter pylori* infection. N. Engl. J. Med. **347:**1175–1186.
24. **van Zwet, A. A., C. M. Vandenbroucke-Grauls, J. C. Thijs, E. J. van der Wouden, M. M. Gerrits, and J. G. Kusters.** 1998. Stable amoxicillin resistance in *Helicobacter pylori*. Lancet **352:**1595.
25. **Versalovic, J., and J. G. Fox.** 2003. *Helicobacter*, p. 915 to 928. *In* P. R. Murray, E. J. Baron, J. H. Jorgensen, M. A. Pfaller, and R. H. Yolken (ed.), Manual of clinical microbiology, 8th ed., vol. 1. American Society for Microbiology, Washington, DC.
26. **Wermeille, J., M. Cunningham, J. P. Dederding, L. Girard, R. Baumann, G. Buri, P. Zelger, J. M. Metry, R. Sitavanc, L. Gallaz, H. Merki, and N. Godin.** 2002. Failure of *Helicobacter pylori* eradication: is poor compliance the main cause? Gastroenterol. Clin. Biol. **26:**216–219.
27. **Wurzer, H., L. Rodrigo, D. Stamler, A. Archambault, T. Rokkas, N. Skandalis, R. Fedorak, F. Bazzoli, E. Hentschel, P. Mora, A. Archimandritis, F. Megraud, et al.** 1997. Short-course therapy with amoxycillin-clarithromycin triple therapy for 10 days (ACT-10) eradicates *Helicobacter pylori* and heals duodenal ulcer. Aliment. Pharmacol. Ther. **11:**943–952.